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9/8/16

Gel from previous day (containing unlabelled minipreps + ligations) was analyzed, found to have nothing of value.

- Parts contaminated?

Instead of ligating ~~ts~~ [pAr lacI ts purp (deg)] + [R011 clpXP CI]
we are now doing:

PAR lacI — ts purp / ts purp LAA / ts purp DAS + R011 clpXP — CI

~~Digest (to make ends sticky)~~

1. ~~Digest~~ digested PAR LacI, ts purp (Q/DAS/LAA), other groups digested backbones (1A3, 1C3, 1K3, 1T3) + R0011-clpXP and CI

Calculations:

Parts were miniprepped at following concentration:

ts purp: 1401 ng/μL
ts purp DAS: 128.4 ng/μL
ts purp LAA: 130.0 ng/μL
PAR LacI: 97.7 ng/μL

We need 1 μg (1000ng) of DNA

ts purp:

$\frac{.1401\ \mu g}{1\ \mu L} = \frac{1\ \mu g}{x\ \mu L}$

$1/.1401 = x$

$7.138\ \mu L = x$

ts purp DAS:

$\frac{.1284\ \mu g}{1\ \mu L} = \frac{1\ \mu g}{x\ \mu L}$

$1/.1284 = x$

$7.788\ \mu L = x$

ts purp LAA:

$\frac{.1300\ \mu g}{1\ \mu L} = \frac{1\ \mu g}{x\ \mu L}$

$1/.1300 = x$

$7.69\ \mu L = x$

PAR lac I

$\frac{.0977\ \mu g}{1\ \mu L} = \frac{1\ \mu g}{x\ \mu L}$

$1/.0977 = x$

$10.235\ \mu L = x$

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amt of water needed -

ts purp: 20 − 7.138 (DNA) − 3 (Enzymes) = 9.862 H_2O
ts purp DAS: 20 − 7.788 (DNA) − 3 (Enzymes) = 9.212 µL H_2O
ts purp LAA: 20 − 7.69 (DNA) − 3 (Enzymes) = 9.31 µL H_2O
P1R LacI: 20 − 10.235 (DNA) − 3 (Enzymes) = 6.765 µL H_2O

Protocol:
1) Do calculations to see how much µL of DNA you need to get 1ug.
2) Determine which enzymes you need
   - Part one (P1R LacI): E and S
   - Part two (ts purp (Q/PAS/LAA): X and P

E and X are remixes, 2µL of that are needed
S and P are standard, 1µL of it is needed
3) Put the ingredients into a microcentrifuge tube in this order: H_2O, DNA, enzymes. You should have 20µL total.
4) Incubate at 37° for 1hr, then at 80° for deactivation
   * Use thermocycler + make diagram of tube placement because ink will wear off *

9/9/16
- parts were ligated:
  - P1R lacI ts purp
  - P1R lacI ts purp LAA
  - P1R lacI ts purp DAS

  } transformations for these were started (10µL of DNA was used)

R11 ClpXP C1 was miniprepped
- concentration: 53.7 ng/µL
- A260/A280: 1.86

9/12/16
transformation results:
- 3 phenotypes on each plate (P1R lacI ts purp Q/LAA/DAS)
  RFP, ts purp, normal

colony PCR on each phenotype

continued from page

Purpose: to prepare biobricks for sequencing

Protocol:

1) calculate amounts of DNA needed:
   - There must be 12μL total – .8μL primer, X DNA + rest
   - 1 μg (1000ng) DNA, so calculate w/ miniprep conc.

pAR lacI: 90.9 ng/μL

$\frac{90.9\text{ng}}{1\mu\text{L}} = \frac{1000\text{ng}}{X\ \mu\text{L}}$

$11.0\mu\text{L} = X$

ts purple ①

$\frac{77.4\text{ng}}{1\mu\text{L}} = \frac{1000\text{ng}}{1\mu\text{L}}$

$12.9\ \mu\text{L}$

ts purple ②

$\frac{364.3\text{ng}}{1\mu\text{L}} = \frac{1000\text{ng}}{X\ \mu\text{L}}$

$2.74\mu\text{L} = X$

ts purp ③

$\frac{120.0\text{ng}}{1\mu\text{L}} = \frac{1000\text{ng}}{1\mu\text{L}}$

$8.33 = X$

* total rxns:

pAR lac I: 11.0μl DNA + .8μl primer + 0.2μL H_2O

ts purple ①:

ts purple ②: 2.7[illegible]μL DNA + .8μL primer + [illegible]8.5μL H_2O

ts purple ③: 8.33μL DNA + .8μL primer + 2.9μL H_2O

2) puncture rubber top with pipet tip. Put ingredients inside
3) Mail using prepaid envelope.

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9/22

transformation of
- DAS ligation w/ IC3 } onto
- LAA ligation w/ IC3 } cam plates

1) Thaw materials on ice for 5 minutes
2) Put 10 µL of ligation onto 100 µL (1 tube) of cells
3) Flick the tube to mix
4) Put on ice for 30 minutes
5) Add 200 µL of LB media / SOC
6) Incubate at 1hr at 37c°
7) Plate 150 µL of cells onto a plate, to a lawn
   ☆MAKE SURE YOU USE PROPER ANTIBIOTIC

- Used C2987, NOT NEB Beta cells

9/23

☆ successful :)

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10/4 : sequencing
eGFP. 3p. F primer (10 μM)

~~tube 1 tube 2~~
~~VI2-VR: R11 ClpXP CI (nd first)~~
tube 3 eGFP : P1R GFP LAA
#4 eGFP P1R GFP DAS
#5 eCFP P1R GFP ~~&~~

Final Plans:
1) P1R GFP & ClpXP CI (48.7 ng/μL)
2) P1R GFP DAS ClpXP CI (297.3 ng/μL)
3) P1R GFP LAA ClpXP CI (285.6 ng/μL)

calculations:
1) $\frac{48.7\text{ng}}{1\mu\text{L}} = \frac{1000\text{ng}}{x\mu\text{L}}$
$20.5\mu\text{L} = x$
no tag

2) $\frac{297.3\text{ng}}{1\mu\text{L}} = \frac{1000\text{ng}}{x\mu\text{L}}$
$3.363 = x\ \mu\text{L}$
DAS
$7.837\ H_2O$

3) $\frac{285.6\text{ng}}{1\mu\text{L}} = \frac{1000\text{ng}}{x\mu\text{L}}$
$3.5\mu\text{L} = x$
LAA
$7.7\ H_2O$

tube AHA7SC: P1R GFP LAA ClpXP CI
tube AHA7S7: P1R GFP DAS ClpXP CI
tube AHA7S3: P1R GFP ~~&~~ ClpXP CI

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Backbone Switch for:

1) PλR LacI (PλRL)
2) ROOII ClpXP CI (RCpCI)
3) PλR LacI GFP LAA ROOII ClpXP CI (pLAA CpCI)
4) PλR LacI GFP DAS RODII ClpXP CI (pDAS CpCI)

1) Digest

(not digesting RCpCI, not enough) conc. 90.9

Amounts:

PλR LacI: 2μL E + 1μL P + 11.0 of DNA + 6 H_2O = 20μL

PLAACpCI: 2μL E + 1μL P + 17.0 of DNA = 20μL

PDASCpCI: 2μL E + 1μL P + 17.0 of DNA = 20μL

Order: Water:
DNA
Enzymes

10/14:

Redigested the above mixtures, due to inconsistant levels of 10/13 miniprep

Ligation Calculation

Parts:

PλR (in 3TS) 4a+10GS (3TS: 3200)
PLAACpCI (in AT3) above + ~~4GS~~ 753 + 2a74 (AT3: 3450)
pDAS CpCI (in AT3) above + 753 + 2a74

Part	length of F	length of V	total		μL
Vector (3)	20	2100	2100	4.7	1
PλR LacI	1114	3200	4314	.23	(ROOII) x3 = 6.12
PLAA	~~829~~ 4841	3450	8291	.12	(3.91) x3 = 11.75
pDAS	~~829~~ 4841	3450	8291	.12	" = 11.75

10/15
transformation of ligation (see above) onto CAM plates
sequencing!

R0011 ClpXP CI in 1AK3, ~~10 beta cells~~ : 175.8 ng/μL (1.85)
P1R LacI cUFP & ClpXP CI in 1C3 : 79.1 ng/μL (1.69)

Calculations:

R11 cpCI:

$$\frac{175.8\ ng}{1\ \mu L} = \frac{1000\ ng}{x\ \mu L}$$

5.68 μL

P1R ... XPCI:

$$\frac{79.1\ ng}{1\ \mu L} = \frac{1000\ ng}{1\ \mu L}$$

12.64 μL

R11: 5.68 μL DNA + .8 prim. + 5.52 H_2O
R1R: 11.2 μL DNA + .8 prim

R0011 ClpXP CI w/ VF2: ADF954
" w/ VR: ADF956

P1R ... CI w/ VF2: ADF953
" w/ eGFP: ADF955
w/ VR: AHA758

10/16
P1R LacI grew, pLaDAS cpCI + pLaLAAcpCI did not.
We are re-digesting + ligating them.

Signature	Date
Witnessed And Understood By	Date